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Research Article

GREEN PHYTO-SYNTHESIS OF GOLD NANOPARTICLES USING *ACHYRANTHES ASPERA LINN* SEED-EPICOTYLS LAYER EXTRACTS AND ITS ANTICANCER ACTIVITY

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ABSTRACT

In the recent past decades, green phyto-process for the synthesis of metal incorporated nanoparticles has been evolving into an imperative branch of nanotechnology. We have reported a rapid, expedient, and extracellular method for the synthesis of gold nanoparticles (AuNPs) by reducing gold chloride with the help of aqueous seed-epicotyls layer extracts of *Achyranthes aspera* Linn *(Amarantheceae).* This approach is simple, economic, stable for a long time, reproducible at room temperature and is synthesized in an eco-friendly mode to obtain a self-assembly of AuNPs. The resulting AuNPs were characterized using ultraviolet-visible absorption spectroscopy, scanning electron microscopy, X-ray diffraction, and Fourier transform infrared spectroscopy spectroscopic techniques. The anticancer activity of the AuNPs was studied against HeLa (Cervical) cancer cell lines. We herein report for the first time, *A. aspera* seed-epicotyls assisted synthesis of biogenic AuNPs; the NPs are conspicuously smaller and better faceted compared with those synthesized by *A. aspera* leaf extracts previously reported. Synthesized AuNPs showed potent anticancer activity at 50 µg/ml concentration against cervical cancer cell lines.

Keywords: Gold nanoparticles, Anticancer activity, *Achyranthes aspera* Linn, Aqueous leaves extract, Scanning electron microscopy, X-ray diffraction, HeLa cancer cell lines.

INTRODUCTION

Past few decades, a significant interest has been focused on metal incorporated nanoparticles (NPs) due to their special properties and impending applications in interdisciplinary fields. NPsNanoparticles, due to their specific electrical, optical, magnetic, chemical, and mechanical properties are currently used in many high technology areas, such as the medical sector for diagnosing chronic diseases, drug delivery, as well as in the field of electronic industry (or) in the field of chemical sector for catalytic reactions, for environmental protection and energy conversion [1-3]. Various chemical methods were established to synthesize gold nanoparticles (AuNPs) and in most of the cases, the reductant and capping agents were toxic in nature. Since AuNPs were used in many human contacting applications, it is necessary to avoid toxic chemical methods and to bring forward eco-friendly green methods to synthesize AuNPs. Apart from chemical reduction, there are many techniques such as laser ablation, aerosol technologies, ultrasonic fields have been used successfully to synthesize AuNPs, but they are expensive. Hence, So developing an inexpensive, eco-friendly, and *in situ* technique which can synthesize AuNPs rapidly is of great interest to current researcher's in nanotechnology. *Achyranthes aspera Linn* belonging to the family *Amarantheceae*, is an erect annual herb, seen growing in the hilly districts of India [4]. The plant is used in indigenous structure of traditional medicine systems as an antibacterial [5], antiviral [6], anticancer [7], antioxidant [8], anti-inflammatory and antiarthritic [9], anti-fertility [10], and antiplasmodic [11]. It is also used in antitumor activities [12,13], in the treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases, and rabies [14,15]. The juice extracted from the root of this plant is mixed along with the root extracts of *Urena lobata* and the bark of *Psidium guajava*, and is used in the treatment of diarrhoea and dysentery [16]. The major phyto-compounds isolated from the plant species is used for the treatment of kidney stone problems [17]. The plant-mediated green synthesis of NPs is gaining importance due to its eco-friendliness and simplicity. Even though synthesis of AuNPs by plants such as *Maduca longifolia* [18], *Aloe vera* [19], *Cinnamomum camphora* [20], *Emblica officianalis* [21], lemongrass [22], neem [23], tamarind [24], *Syzygium aromaticum* [25], have been reported, the potential of plant extracts as reductant/ surfactants for the synthesis of NPs is yet to be fully explored. Recently, using the leaf extracts of *A. aspera* synthesize of Ag and AuNPs nanoparticles was reported [26, 27]. In the previously reported method, the time compatibility and quantification process of AuNPs was too large, but in our reported method, the time management and quantification process of seed epicotyls layer extract AuNPs is less.

MATERIALS AND METHODS

Collection of plant materials and AuNPs phyto-synthesis

Fresh seed of *A. aspera* Linn was identified and collected from Vellore district, Tamilnadu, India and the taxonomic identification was made by ABS Botanical Garden, Salem. The specimen was numbered and kept in our research laboratory for further reference. Chloroauric acid (HAuCl4) was purchased from Sigma-Aldrich, India.

Preparation of *A. aspera* seed epicotyls layer extract

The fresh *A. aspera* Linn seeds were collected from surroundings of Vellore district, Tamilnadu, India. The seed epicotyls layer extract is used for the reduction of Au^{3+} ions to AuNPs. 1 g of finely grinded and meshed *A. aspera* seed powder was mixed with 100 ml of deionized water and heated at 90°C on temperature controlled water bath for about 1 hr and cooled, passed through 0.2 µm cellulose nitrate membrane filter paper.

Synthesis of AuNPs

The aqueous seed epicotyls layer extract of 200 µl of *A. aspera* Linn was added to 2 ml of 0.01 M HAuCl4 solution and mixed thoroughly by manual shaking. Formation of AuNPs was instantaneous and noticed by visual color change from yellow to pinkish red.

Characterization of the synthesized AuNPs

Ultraviolet (UV) visible spectroscopy

The initial characterization of synthesized AuNPs was carried out using UV-visible spectroscopy. The reduction of gold ions was monitored from 400 to 1350 nm on Hitachi double beam spectrophotometer (model U-2800) after 5-fold diluting the sample with deionized water against deionized water as blank. The recorded spectral data were then plotted using Origin 6.1.

X-ray diffraction (XRD)

Colloidal AuNPs was purified after centrifugation at 15,000 rpm and then subjected to XRD analysis (Bruker D8 Advance diffractometer with Cu Kα radiation, λ=1.54Å). The scanning range was done between 30° and 90°. Lanthanum hexaboride was used to calibrate the instrument before analysis.

Fourier transformed infrared spectroscopy (FT-IR)

A purified AuNPs in the form of powder obtained from the seed epicotyls layer extract was analyzed using FT-IR spectroscopic measurement. The measurements were carried out on THERMO NICO LET AVATAR 330 FT-IR 4000-400/cm in KBr pellets.

Potentiometric study

Oxidation-reduction potential changes of nanomaterials in broth with time were studied using a potentiometer (Equip-Tronics dual channel potentiometer model EQ-603) with a combination of the platinum electrode and saturated calomel electrode.

Field emission scanning electron microscopy (FESEM)

The morphology and the size of the AuNPs particles were examined with FESEM (Hitachi SU6600) by an accelerating voltage of 15 kV. The spot size in FESEM is smaller than in the conventional scanning electron microscopy (SEM), and it can produce very high resolution images (better than 3-6 times of the conventional SEM). The identification of elemental composition of the AuNPs was done using EDX spectrum which is also recorded with FESEM instrument.

Anticancer activity

3-(4,5-dimethythiazol 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [28,29]-(cervical cancer cell lines) is obtained from ATCC and maintained in DMEM (Hi-Media Laboratories Pvt. Ltd, Mumbai, India) supplemented with 10% heat-inactivated fetal bovine serum (v/v), streptomycin (100 µg/ml) and penicillin (100 µg/ml). The cell line was maintained at 370°C with 5% carbon dioxide in CO_2 incubator. The MTT cell proliferation assay is used to evaluate the anticancer activity of the synthesized AuNPs using the cell quantification MTT cell viability assay kit (Bioassay Systems). The optical density is measured at 570 nm for each well on the absorbance plate reader. Trypan blue dye exclusion assay was also used to count the number of viable and non-viable HeLa cancer cells in the culture medium after drug treatment. Treatment with cisplatin at the same concentration serves as a positive control.

RESULTS AND DISCUSSION

Mechanism of AuNPs formation using seed epicotyl layer extract

In the reduction process, where gold ions are converted into AuNPs was monitored using a potentiometeric experiment. There was a sharp decrease in potential within 5 seconds of interaction between chloroauric acid and *A. aspera* seed epicotyl layer extract which indicated the instantaneous formation of AuNPs. The potential was decreased to 0.062 V from the initial potential of gold ions (0.589 V) at the end of 20 seconds. After completion of the NPs green synthesis, the potential of the solution remained constant as shown in Fig. 1. UV-visible spectral data also confirmed the instant conversion of Au ions to AuNPs within a few seconds.

The formation of AuNPs was confirmed by visual color change simultaneously in UV-visible spectra as shown in Fig. 2. The formation of AuNPs was obtained *in situ* i.e. when the *A.aspera* seed epicotyls layer extract was added to the chloroauric acid solution, immediately the color changed from yellow to pinkish red which indicated the formation of AuNPs. The UV-visible spectrum shows two bands-one in the visible region around 539 nm and another broadband in the NIR region which is an indicative of the formation of anisotropic effect of NPs.

The presence of polyphenolic biomolecules in *A. aspera* seed epicotyls layer extract and their interaction with the surface of the AuNPs was confirmed by FT-IR spectra. The IR bands were observed at 3629, 2971, 2923, 1449, 1381, 1164, 1069, 1022/cm in dried *A. aspera* seed epicotyls layer extract powder are characteristics of O-H, -C-H, stretching modes of the hydroxyl group present in the phenolic and tannins. The bands at 1654 and 1639/cm are assigned to the C=O stretch of the acid group

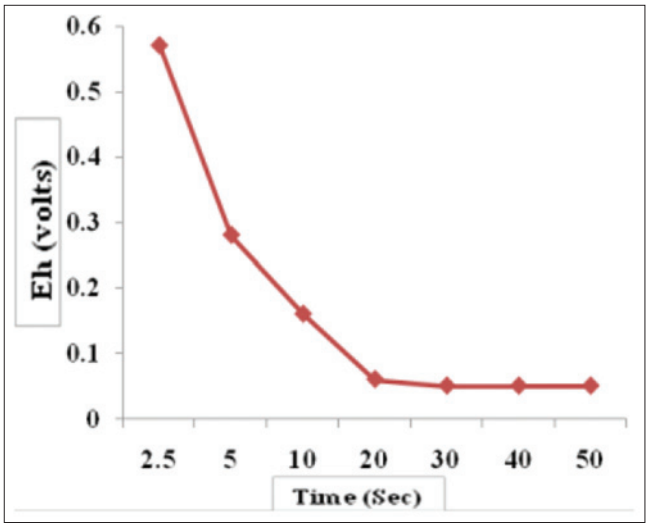

Fig. 1: The formation of gold nanoparticles from $HAuCl_4$ aqueous solution using *Achyranthes aspera* seed extract with time by potentiometer

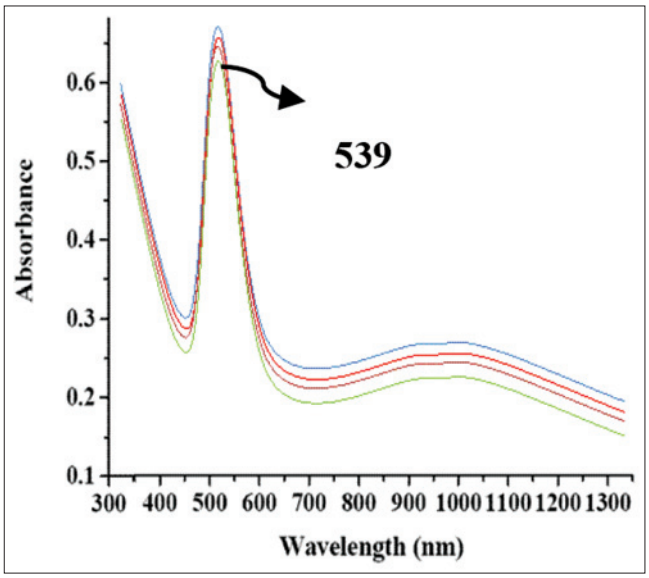

Fig. 2: The formation of gold nanoparticles from $HAuCl_4$ aqueous solution using *Achyranthes aspera* seed epicotyls layer extract - ultraviolet-visible spectra

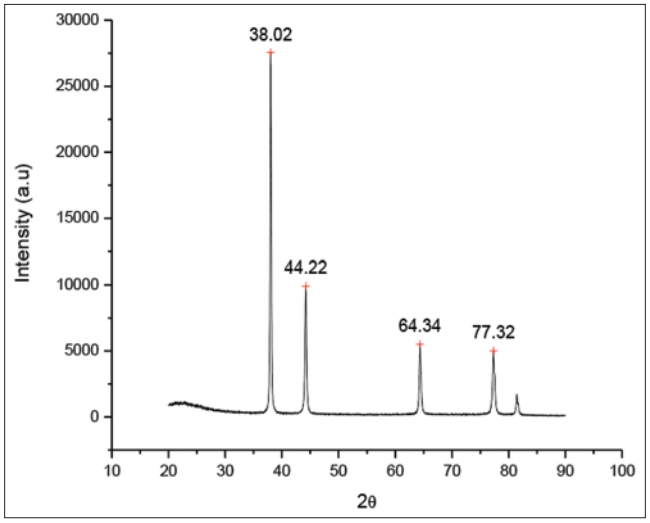

Fig. 3: X-ray diffraction spectrum - The formation of gold nanoparticles from $HAuCl_4$ aqueous solution using *Achyranthes aspera* seed epicotyls layer extract

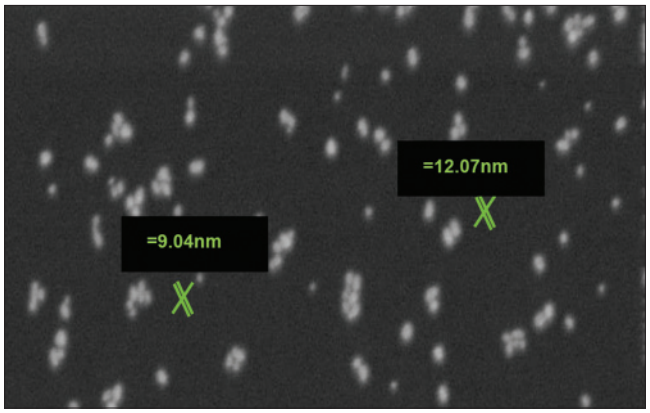

Fig. 4: The formation of gold nanoparticles from $HAuCl_4$ aqueous solution using *Achyranthes aspera* seed epicotyls layer extract - field emission scanning electron microscopy

present in the phenolic and tannic acid present in *A.aspera* powder. Except for a slight shift in the C=O stretching bonds 1654-1648/cm and 1639-1631/cm and rest of the IR bands remain unchanged in the spectra obtained from that AuNPs after the reaction with *A.aspera* seed epicotyls layer extract.

The XRD patterns of vacuum-dried AuNPs is synthesized using aqueous seed-epicotyls layer extracts of *A. aspera Linn* as shown in Fig. 3. The XRD pattern of Au^{3+}-Au^{+}/*A. aspera* Linn indicates that the structure of AuNPs is face-centered cubic (FCC).

The XRD peaks for the synthesized AuNPs at 2θ of 38.02, 44.22, 64.34, and 77.32 can be predictable with crystallographic planes of 111, 200, 220, 311 confirms FCC gold crystals, respectively. The lattice plane of FCC AuNPs (Literature Report - JCPDS 04-0783) as shown in the Fig. 3.

FESEM

FESEM images were obtained using FESEM ∑ (Sigma), (Hitachi SU6600). The FESEM image (Fig. 4) of the AuNPs in sample S4 confirmed that the particles are irregular spherical, hexagonal, triangular, and elongated shapes. The FESEM images of particles synthesized through the mixed solvent system (95 vol. %) at various magnifications. From these images, it is clear that the particles have the morphology of spherical shape and the size in the range of 20-30 nm. In this case the particles

Table 1: Anticancer activity of green synthesized AuNPs

Compound	Concentartion (µg/ml)	HeLa cancer cell lines		
		Absorbance (OD)±STD	IC_{50}	r^2
AuNPs	50	1.93±0.002	0.027	0.92
	40	1.80±0.003		
	30	1.49±0.002		
	20	1.45±0.003		
	10	1.32±0.002		
Standard (cisplatin)	50	2.38±0.002	0.023	0.95
	40	2.15±0.002		
	30	1.97±0.002		
	20	1.80±0.002		
	10	1.64±0.002		

IC_{50}: Inhibitory concentration 50, AuNPs: Gold nanoparticles

sizes are slightly increased and is also observed that the particles are homogeneously distributed without agglomeration compared to that of the former case.

The anticancer activity of AuNPs was evaluated using MTT assay method. *In vitro* cervical cancer cell lines were prepared at different concentrations (10, 20, 30, 40, and 50 µg/ml). The results of the anticancer activity are shown in Table 1 at different concentrations were analyzed and at lower concentration of 25-30 µg/ml AuNPs showed a significant anticancer effect. The anticancer activities of the AuNPs at five different concentrations were compared with that of the standard anticancer drug cisplatin.

CONCLUSION

A facile rapid green eco-friendly and one-step method is used to synthesize AuNPs via aqueous seed-epicotyls layer extracts of *A. aspera* Linn. Polyphenols were present as the major phytoconstituents in the aqueous seed extracts of the plant, which will serve both as reducing and capping agents. This method is a promising alternative to the traditional reduction routes to avoid usage of toxic chemicals. AuNPs obtained by this method exhibits significant anticancer activity against cervical cancer cell lines. This green method may find various medicinal as well as technological applications in demands.

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